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TITLE: Maintenance of Glucose Homeostasis through Acetylation of the Metabolic Transcriptional Coactivator PGC-1alpha

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14. ABSTRACT

The main purpose of this proposal is to test the hypothesis that acetylation of PGC-1α by GCN5 and associated proteins, Pc3 and WDR18, is a key regulatory modification that controls hepatic glucose production. The major findings of this Research Technical Report are in tasks 1, 2 and 3. In task 1, we have further validated siRNAs for Pc3 and WDR18. In task 2, we have identified how WDR18 specifically interacts with GCN5 and does not interfere with the formation of the PGC-1α transcriptional protein complex. In Task 3, in order to identify what specific lysines are important for the PGC-1α function, we have also mapped the PGC-1β lysines that are acetylated by GCN5. These experiments have pointed out to specific sites on PGC-1α that are also acetylated and might control hepatic glucose output. We will continue to complete the proposed tasks to understand how PGC-1α acetylation controls hepatic glucose production through the GCN5 complex.

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Maintenance of Glucose Homeostasis Through Acetylation of the Metabolic Transcriptional Coactivator PGC-1α.

Abstract (160 words)
The main purpose of this proposal is to test the hypothesis that acetylation of PGC-1α by GCN5 and associated proteins, Pc3 and WDR18, is a key regulatory modification that controls hepatic glucose production. The major findings of this Research Technical Report are in tasks 1, 2 and 3. In task 1, we have further validated siRNAs for Pc3 and WDR18. In task 2, we have identified how WDR18 specifically interacts with GCN5 and does not interfere with the formation of the PGC-1α transcriptional protein complex. In Task 3, in order to identify what specific lysines are important for the PGC-1α function, we have also mapped the PGC-1β lysines that are acetylated by GCN5. These experiments have pointed out to specific sites on PGC-1α that are also acetylated and might control hepatic glucose output. We will continue to complete the proposed tasks to understand how PGC-1α acetylation controls hepatic glucose production through the GCN5 complex.

Introduction
Homeostatic mechanisms in mammals, including humans, function to maintain blood glucose levels within a narrow range in response to hormones and nutrients. For example, high stress and intense exercise conditions combined with food deprivation make soldiers very vulnerable to changes in blood glucose levels. Glucose homeostasis is dysregulated in metabolic diseases such as obesity and diabetes which have high incidence in the US population. We study a biochemical process that controls blood glucose levels through control of hepatic glucose synthesis. This regulatory control is achieved by a chemical modification –acetylation- of the PGC-1α metabolic transcriptional coactivator (Rodgers et al. 2005) (Lerin et al. 2006). The main purpose and scope of this Research Proposal is to decipher how two proteins, Pc3 and WDR18, that control the enzymatic activity of the PGC-1α GCN5 Acetyl Transferase regulate PGC-1α acetylation and its effects on glucose metabolism. We are using biochemical and physiological approaches, both in cell culture and mouse models, to precisely identify the key acetylation sites on PGC-1α that are required and sufficient to modulate blood glucose levels. These findings have strong implications for the basic pathways of energy homeostasis, diabetes and metabolic diseases and will certainly benefit performance of personnel in the army that work in conditions of high stress.

Body
In this body section of this Research Technical Report, we will describe in detail the experimental data and interpretations of the results obtained in the second year of the award. As previously stated in the SOW we have performed experiments that were proposed in Tasks 1, 2, and 3. We have mainly accomplished the goals that were originally stated and based on those results, we will continue the Tasks that were proposed.

Task 1. Analysis of two novel proteins in the GCN5 complex (Pc3 and WDR-18) that strongly repress PGC-1α glucose production function. Importantly, WDR-18 is regulated by nutritional status and in diabetic models. (Months 1-24).

• Test of different siRNAs (for Pc3 and WDR-18) on PGC-1α function. Transcriptional Analysis using luciferase reporter systems. (Months 12-24).

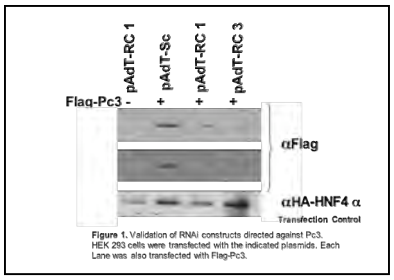

Figure 1. Validation of RNAi constructs directed against Pc3. HEK 293 cells were transfected with the indicated plasmids. Each Lane was also transfected with Flag-Pc3.

In the previous Research Technical report we incorporated some of the functional analysis on PGC-1α transcriptional activity with different RNAis generated against Pc3 and WDR-18. Although at that time we did not known the efficiency of efficiency for Pc3, but increases on PGC-1α activity were detected –see 2007 Report-; in Fig. 1 we show that the RNAis used produced a significant knock-down on Pc3 protein expression levels. We have produced adenoviruses encoding for those specific RNAis. Thus, we are now in a position to test the effect of these RNAis on endogenous gluconeogenic gene expression and hepatic glucose output that will be performed as previously described in Task 4.

Although in the previous report we described an RNAi against WDR-18 that was sufficient to increase the PGC-1α transcriptional activity, in this term we have generated additional RNAis to optimize and select the one with strongest knock-down effects. As shown in Fig. 2B, two specific RNAis were further designed against WDR-18 (RW1 and RW2). RW1 had a significant decrease in WDR18, however RW2 completely knocked down the expression of this protein in transient transfection experiments. We have therefore decided to use RW2 as an efficient RNAi against WDR-18. To further demonstrate that WDR-18 knock down had an effect on PGC-1α transcriptional activity, we used again the relevant pro-gluconeogenic transcription factor HNF4α which is strongly coactivated by PGC-1α. Fig. 2A shows that PGC-1α transcriptional coactivation on HNF4a was increased when WDR-18 was knocked-down. We have now produced adenoviruses that efficiently express this hairpin and decrease WDR-18 levels. Again, and similar with Pc3, we are in a unique position to test the effects of WDR-18 on expression of gluconeogenic genes and hepatic glucose output. This constitutes our next experiments that were detailed in Task 4.

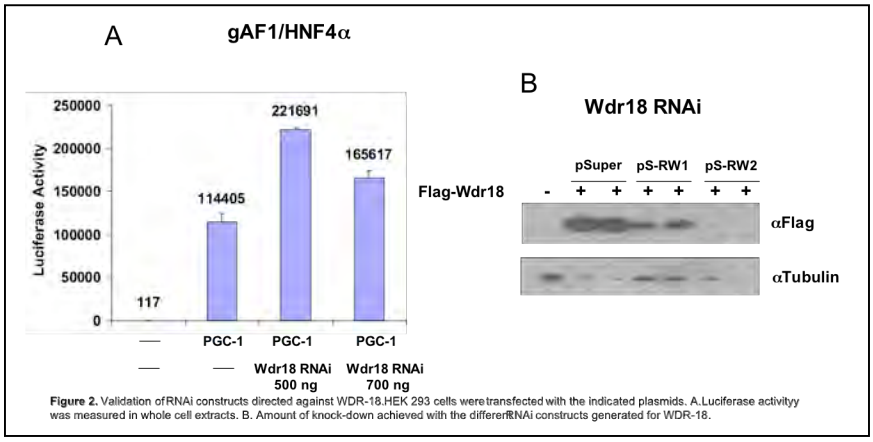

Figure 2. Validation of RNAi constructs directed against WDR-18.HEK 293 cells were transfected with the indicated plasmids. A.Luciferase activityy was measured in whole cell extracts. B. Amount of knock-down achieved with the differentRNAi constructs generated for WDR-18.

Task 2. Identification of the mechanisms of PGC-1α's repression by acetylation and interaction with GCN5-associated proteins Pc3 and WDR-18. (Months 12-36).

• Analysis of PGC-1α cellular nuclear localization modulated by Pc3 and WDR-18. Microscopy immunofluorescence specific antibodies. (Months 12-18).

1. PGC-1α nuclear localization regulated by GCN5. We have previously shown that expression of GCN5 re-localizes PGC-1α from the nucleoplasm to rounded foci. In those nuclear structures PGC-1α is largely inactive and co-localizes with the nuclear hormone receptor repressor RIP-140 (Lerin et al. 2006). Since we observed that Pc3 as well as WDR-18 increased PGC-1α acetylation and decrease its transcriptional activity, we decided to analyze the nuclear distribution of PGC-1α both in gain- and loss-of-function experiments. In control experiments, we clearly reproduce the effects of GCN5 to re-localize PGC-1α to nuclear foci. However, overexpression or knock-down of Pc3 as well as WDR-18 did not significantly altered PGC-1α nuclear sublocalization –data not shown-. Thus, it appears that the mechanisms by which Pc3 and WDR-18 control PGC-1α transcriptional activity are not through nuclear localization. Interestingly, we have previously reported that GCN5 also blocks the recruitment of PGC-1α to chromatin promoters of gluconeogenic genes (Lerin et al. 2006). Thus, in this task, -see above- we will investigate whether the mechanisms of action of Pc3 and WDR-18 is by modulating the ability of PGC-1α to be recruited at promoter regions.

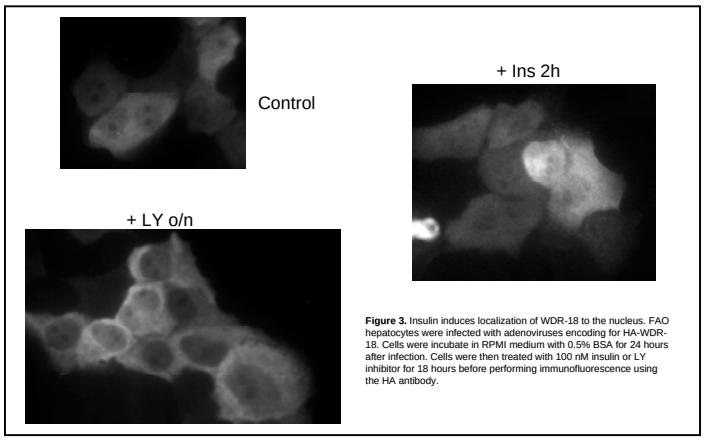

Figure 3. Insulin induces localization of WDR-18 to the nucleus. FAO hepatocytes were infected with adenoviruses encoding for HA-WDR-18. Cells were incubate in RPMI medium with 0.5% BSA for 24 hours after infection. Cells were then treated with 100 nM insulin or LY inhibitor for 18 hours before performing immunofluorescence using the HA antibody.

2. Insulin-regulated WDR-18. Interestingly, while we were performing the nuclear localization experiments we observed that WDR-18 localizes differently between the cytoplasm and nucleus dependent on the insulin signaling. It is important to note that insulin is the main hormone that represses gluconeogenic genes under the fed state (Pilkis and Granner 1992). As shown, in Fig. 3, inhibition of PI3K with the LY inhibitor, WDR-18 is exclusively localized in the cytoplasm, however, after insulin stimulation WDR-18 is WDR-18 is transported to the nucleus. Although, this is not proposed in this proposal, this regulation might provide a mechanism by which WDR18 is regulated. Our current model is that under insulin stimulation, WDR18 translocates to the nucleus where it physically binds to GCN5 protein complex. In the nucleus, WDR18 binds to the catalytic domain of GCN5 –see below- and activate its acetyl transferase enzymatic activity on PGC-1α. Acetylated PGC-1α is inactive and cannot activate gluconegeonic genes and hepatic glucose output.

• Analysis of physical interaction between PGC-1α, transcriptional regulatory proeins that are known to conctrol gluconeogenic genes and two novel proteins in

the GCN5 protein complex (Pc3 and WDR-18). Immunoprecipitation analysis (Months 18-30).

1. Pc3 interacts with GCN5 in an insulin-independent manner. Our previous analysis indicated that Pc3 was identified in the PGC-1α protein complex. To further validate the physical interaction between Pc3 and GCN5 we performed immunoprecipitation analysis. Fig.4 shows that GCN5 interacts with Pc3 and that this interaction is not influenced by the presence of insulin. Thus, in contrast to WDR-18 the interaction with of Pc3 and GCN5 is not affected by this signaling. This would suggest that perhaps other mechanisms to regulate the activity of GCN5 take place. In this task, our next experiments will be devoted to analyze whether Pc3 also interacts with PGC-1α and whether this interaction might be regulated.

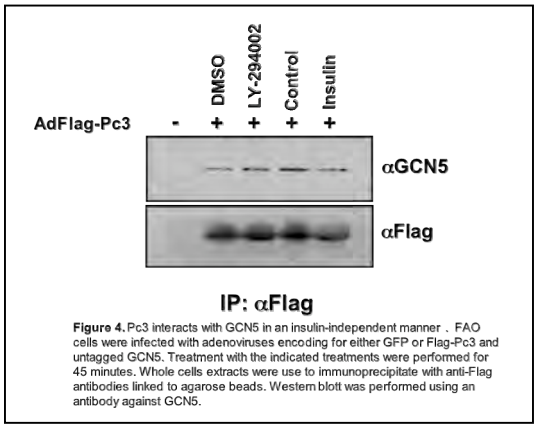

Figure 4. Pc3 interacts with GCN5 in an insulin-independent manner . FAO cells were infected with adenoviruses encoding for either GFP or Flag-Pc3 and untagged GCN5. Treatment with the indicated treatments were performed for 45 minutes. Whole cells extracts were use to immunoprecipitate with anti-Flag antibodies linked to agarose beads. Western blott was performed using an antibody against GCN5.

2. WDR-18 does not directly interact with PGC-1α but it does with GCN5. Our previous analysis indicated that WDR-18 was in the PGC-1α complex. Consistent with this data we found that PGC-1α as well as GCN5 were able to immunoprecipitate WDR-18 (Fig. 5C). In order to determine whether WDR-18 directly interacts with GCN5 and/or PGC-1α, we performed in-vitro interaction analysis using GST-fusion proteins. Fig. 5 shows that WDR18 does not directly interact with PGC-1α, however it does with GCN5. This data strongly indicate that WDR18 is present in the GCN5 subcomplex of the global PGC-1α complex. To map the interaction domain between WDR-18 and GCN5, we made different GCN5 constructs encoding for fusion proteins. Fig. 5b shows that WDR18 directly interacts with the catalytic domain of GCN5. Based on these results, it is possible that the binding of WDR-18 affects the enzymatic activity of GCN5. Thus, by modulating this enzymatic activity it would controls PGC-1α transcriptional activity.

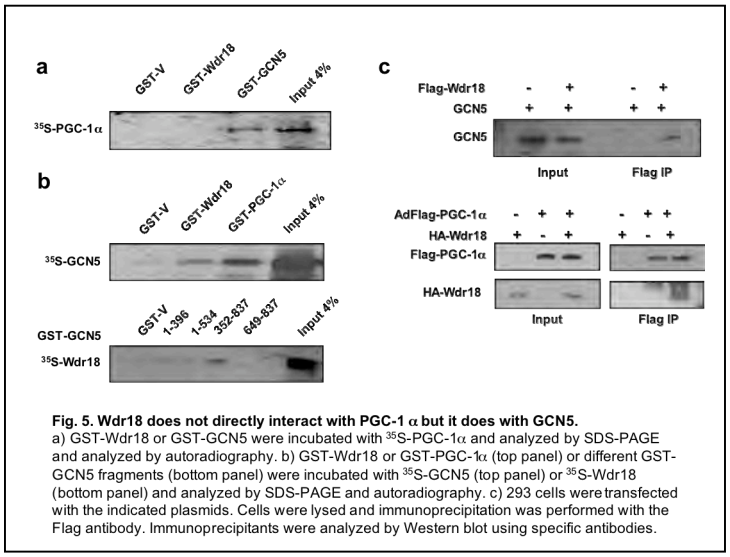

Fig. 5. Wdr18 does not directly interact with PGC-1 α but it does with GCN5.
a) GST-Wdr18 or GST-GCN5 were incubated with ^{35}S-PGC-1α and analyzed by SDS-PAGE and analyzed by autoradiography. b) GST-Wdr18 or GST-PGC-1α (top panel) or different GST-GCN5 fragments (bottom panel) were incubated with ^{35}S-GCN5 (top panel) or ^{35}S-Wdr18 (bottom panel) and analyzed by SDS-PAGE and autoradiography. c) 293 cells were transfected with the indicated plasmids. Cells were lysed and immunoprecipitation was performed with the Flag antibody. Immunoprecipitants were analyzed by Western blot using specific antibodies.

3. WDR-18 interacts with the GCN5 susbtrate histone 3. To further identify key proteins of the chromatin dynamics that were important for WDR-18, we decided to analyze whether the GCN5 substrate histone H3 interacts with WDR-18. Fig. 6 that WDR-18 interacts with WDR-18 using immunoprecipitation analysis. These experiments suggest

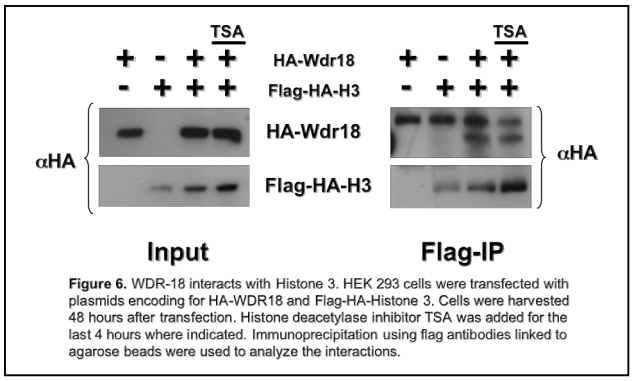

Figure 6. WDR-18 interacts with Histone 3. HEK 293 cells were transfected with plasmids encoding for HA-WDR18 and Flag-HA-Histone 3. Cells were harvested 48 hours after transfection. Histone deacetylase inhibitor TSA was added for the last 4 hours where indicated. Immunoprecipitation using flag antibodies linked to agarose beads were used to analyze the interactions.

that WDR-18 is part of a transcriptional complex at the chromatin of promoter genes. The fact that WDR-18 contains several WD-40 repeats suggests that it uses those domains as docking sites for different proteins in this transcriptional complex including GCN5 and histone H3.

• *Chromatin Immunoprecipitation Analysis. Determination of PGC-1α chromatin occupancy on gluconeogenic genes depending on GCN5 activation modulated by association with Pc3 and WDR-18 (Months 18-36).*

The fact that Pc3 and WDR-18 were identified as protein components of the PGC-1**α** transcriptional complex (Lerin et al. 2006) and that were specifically part of the GCN5 complex, prompted us to determine their specific association with the chromatin promoters. To this end, first, we have optimized the chromatin immunoprecipitation assays. Fig. 7 shows that ectopic expression of PGC-1**α** with a Flag-tagged can be immunoprecipitated with the chromatin of several target genes. We are now in the position to test whether Pc3 and WDR-18 can influence the ability of PGC-1**α** to be recruited to those promoters. As proposed in the initial task 2, we will use cultured hepatocytes to express adenoviruses encoding for PGC-1**α**, Pc3 and WDR-18 in the presence and absence of GCN5. We have published (Lerin et al. 2006) that GCN5 is sufficient to displace PGC-1**α** from the chromatin promoters. The idea in this subtask is to determine whether Pc3 and WDR-18 by modulating the enzymatic activity of GCN5 are sufficient to alter the association between PGC-1**α** and the chromatin of target gluconeogenic genes. WDR-18 interacts with histone 3 suggesting that it is strongly associated with the chromatin. It would be of interest to determine whether this association with H3 and GCN5 modulate PGC-1**α** chromatin interaction. Those experiments will constitute part of our new tasks in the next year of this grant.

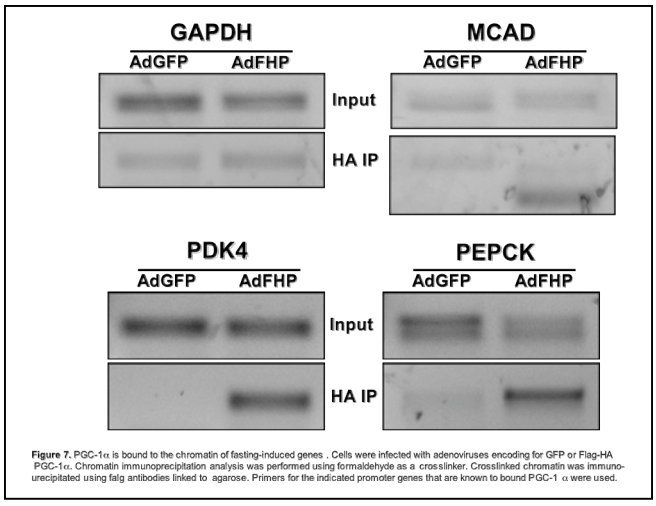

Figure 7. PGC-1α is bound to the chromatin of fasting-induced genes . Cells were infected with adenoviruses encoding for GFP or Flag-HA PGC-1α. Chromatin immunoprecipitation analysis was performed using formaldehyde as a crosslinker. Crosslinked chromatin was immuno-urecipitated using falg antibodies linked to agarose. Primers for the indicated promoter genes that are known to bound PGC-1 α were used.

Task 3. Identification of the important functional acetylated lysines involved in PGC-1α repression. (Months 1-24).

• *Transcriptional analysis of PGC-1α acetylated mutants and regulation by GCN5 associated proteins Pc3 and WDR-18. Analysis using luciferase reporter systems (Months 6-24).*

As described in the previous Scientific Technical Report, the R13 PGC-1α acetylation mutant was still acetylated by GCN5. This data suggested that other lysines are acetylated and might be important for PGC-1α function. In order to identify new potential sites that were acetylated on PGC-1α, we decided to identify lysines that were also acetylated by GCN5 on PGC-1β. The rationale behind those experiments was that similar to PGC-1α, PGC-1β was also strongly inhibited by GCN5, but not by GCN5 acetyltransferase defective mutant (Lerin et al. 2008; Submitted manuscript). Thus, the idea was that perhaps some conserved sites between both transcriptional coactivators might lead to a better understanding of the PGC-1α acetylation function. First, we expressed PGC-1β and GCN5 in cells using adenoviruses that encode both proteins. 12 hours before harvesting, cells were treated with nicotinamide (a SIRT1 deacetylase inhibitor). Nuclear extracts were used to immunoprecipitated PGC-1β using Flag antibodies linked to agarose beads. The PGC-1β band in an SDS-PAGE gel was excised and acetylation sites were identified by LC/MS. Fig. 8 shows the lysines that were identified to be acetylated on PGC-1β. Interestingly, several lysines that were previously identified on PGC-1α (Rodgers et al. 2005) were also found to be acetylated on PGC-1β. However, we decided to focus on K218, which is conserved on PGC-1α is K211 –which is not mutated in the R13-. The reason is because, this K is in the middle of the alpha-helix (LLKYL) and it has been shown to be important for interaction with ERRα –a transcription factor that mediates the PGC-1α mitochondrial function- (Kallen et al. 2004). Based on our and those studies, we will test whether this K acetylation is important for the repression mediated by GCN5. The molecular basis is that this K makes a hydrogen bond with a E that is next to the L in the alpha helix. Thus, the addition of an acetyl group to this K will block the formation of this hydrogen bond.

A.

Acetylation Site	Peptide Sequence
K202	K.KTPTLR.A
K218	R.AQSRPCTELHKHLTSVLPCPR.V
K230	R.VKACSPTPHPSPR.L
K455	W.GRKRPGRGLPW.T
K469	R.KMDSSVCPVR.R
K645	K.LSPGQDTAPSLPSPEALPLTATPGASHKLPK.R
K674	V.SQAGQKRPF.S
K726	R.SWEPIGVHLEDLAQQGAPLPTETKAPR.R
K933	R.GQKHGFITFR.C
K994	R.YTDYDPTSEESLPSSGKSK.Y

B.

MAGNDCGALLDEELSSFFLNYLSDTQGGDSGEEQLCADLPELDLSQLDASDFDSATCFGELQWCPETSETEPSQYSPDDSELFQIDSENEALLAALTKTLDDIPED
DVGLAAFPELDEGDTPSCTPASPAPLSAPPSPTLERLLSPASDVDELSLLQKLLLATSSPTASSDALKDGATWSQTSLSSRSQRPCVKVDGTQDKKTPTLRAQSRP
CTELHKHLTSVLPCPRVKACSPTPHPSPRLLSKEEEEEVGEDCPSPWPTPASPQDSLAQDTASPDSAQPPEEDVRAMVQLIRYMHTYCLPQRKLPQRAPEPIPQA
CSSLSRQVQPRSRHPPKAFWTEFSILRELLAQDILCDVSKPYRLAIPVYASLTPQSRPRPPKDSQASPAHSAMAEEVRITASPKSTGPRPSLRPLRLEVKRDVNKPT
RQKREEDEEEEEEEEEEEEEKEEEEEEWGRKRPGRGLPWTKLGRKMDSSVCPVRRSRRLNPELGPWLTFTDEPLGALPSMCLDTETHNLEEDLGSLTDSSQGR
QLPQGSQIPALESPCESGCGDTDEDPSCPQPTSRDSSRCLMLALSQSDSLGKKSFEESLTVELCGTAGLTPPTTPPYKPMEEDPFKPDTKLSPGQDTAPSLPSPEA
LPLTATPGASHKLPKRHPERSELLSHLQHATTQPVSQAGQKRPFSCSFGDHDYCQVLRPEAALQRKVLRSWEPIGVHLEDLAQQGAPLPTETKAPRREANQNCDP
THKDSMQLRDHEIRASLTKHFGLLETALEGEDLASCKSPEYDTVFEDSSSSSGESSFLLEEEEEEEEGGEEDDEGEDSGVSPPCSDHCPYQSPPSKASRQLCSRS
RSSSGSSSCSSWSPATRKNFRRESRGPCSDGTPSVRHARKRREKAIGEGRVVYIRNLSSDMSSRELKKRFEVFGEIVECQVLTRSKRGQKHGFITFRCSEHAALS
VRNGATLRKRNEPSFHLSYGGLRHFRWPRYTDYDPTSEESLPSSGKSKYEAMDFDSLLKEAQQSLH

Fig. 8. Analysis and identification of PGC-1β lysine acetylation sites. HEK 293 cells were infected with adenoviruses encoding for FLAG-HA-PGC-1β and FLAG-GCN5 and treated with nicotinamide (20 mM) for 12 hours. After immunoprecipitation, PGC-1β was separated by SDS-PAGE and analyzed by tandem mass spectrometry. Acetylation was determined by subjecting a tryptic and a chymotryptic digest of the protein to microcapillary liquid chromatography tandem mass spectrometry (LC-MS/MS) on a hybrid linear ion trap/FT-ICR mass spectrometer (LTQ FT) and assigning the acquired MS/MS data using the SEQUEST algorithm as decribed in the Experimental Section. A. Sequences of identified acetylated peptides including flanking amino acid residues. Acetylated lysine residues are shown in red. B. 87 % of the amino acid sequence (74 % of lysine residues) of PGC-1β was covered in the acetylation mapping experiment (covered residues are shown in green, acetylated lysine residues in red).

In the next 12 months we will extent this task will and we will investigate the effects of this lysine on GCN5-mediated repression of the transcriptional coactivator PGC-1α. At this point to identify these new acetylated lysines is essential to fully accomplish the other tasks. Therefore, our major efforts will be devoted to perform the proposed experiments.

Key Research Accomplishments

In this second year, we have accomplished experiments included in tasks 1, 2 and 3 that were originally proposed in the application.

Task 1.- • We have further tested the effects of knocking down Pc3 and WDR-18 proteins using these siRNAs. In particular with WDR-18, we have generated adenoviruses encoding for an specific siRNA that strongly decreases expression of WDR-18. In luciferase-based assays, knock down of both Pc3 as well as WDR-18 are sufficient to increase the transcriptional activity of PGC-1α.

Task 2.- • We have specifically identified how Pc-3 and WDR-18 specifically interacts with GCN5 and does not interfere with the formation of the PGC-1α transcriptional protein. In the PGC-1α transcriptional complex, both Pc3 and WDR-18 are part of the GCN5 complex and directly interact with GCN5. WDR-18 interacts with the catalytic domain of GCN5 and also interacts with its substrate histone H3. WDR-18 cellular localization is controlled by insulin that induces its translocation to the nucleus to repress gluconeogenic gene expression. Optimization experiments have been performed with Chromatin immunoprecipitation to detect binding of PGC-1a to promoter regions of fasted-induced genes.

Task 3.- • Based on new data on PGC-1β acetylation sites, We have identified new acetylation sites on PGC-1α that are acetylated by GCN5. Importantly, a new site in the third LxxLL motif seems to play an important role in the GCN5-mediated repression of PGC-1α transcriptional activity.

Reportable Outcomes

Task 1, 2 and 3. The results that are described in detail in this Research Technical Report have been partially published and presented in the following conferences or seminars as invited speaker in 2007 and 2008.

J.T. Rodgers and P. Puigserver. Fasting-Dependent Glucose and Lipid Metabolic Response through Hepatic SIRT1. *Proc. Natl. Acad. Sci. USA*. 2007 104(31) 12861-12866.

C. Lerin, T.J. Kelly, W. Haas, S.P. Gygi, and P. Puigserver. GCN5-Mediated Transcriptional Control of the Metabolic Coactivator PGC-1β through Lysine Acetylation. Submitted, 2008.

J. T. Rodgers, C. Lerin, Z. Gerhart-Hines and P. Puigserver. Metabolic Adaptations Through PGC-1α and SIRT1 Pathways. *FEBS Letters*. 582: 46-53. 2008

- University of Utah. Department of Biochemistry. Salt Lake City, UT.
- NIH/NIDDK. Bethesda, MD.
- The Paul F. Glenn Symposium on Aging. Harvard Medical School.
- American Aging Association- 36th Annual Meeting. San Antonio, TX.

- FASEB Summer Research Conferences. Glucose Transporter Biology. Snowmass Village, CO.
-17th Annual Irwin M. Arias, MD Symposium. American Liver Foundation. Boston, MA.
- Nuclear Receptors in Liver and Digestive Diseases: A Research Workshop. Rockville, MD.
- University of Cincinnati. Genomic Research Institute. Cincinnati, OH.
- Keystone Symposia. Diabetes Mellitus, Insulin Action and Resistance. Breckenridge, Colorado.

Conclusion

We have summarized in this Research Technical Report the experiments and results that have been obtained in the second year of this Award. Importantly, we have accomplished the original tasks that were proposed. We have generated additional siRNAs for Pc3 and WDR-18 (we have at this moment adenoviruses for both gain- and loss-of-function) that will allow us to accomplish the next tasks that were proposed in SOW. In addition, we have obtained important information about the nature of interaction of Pc3 and WDR-18 in the PGC-1α transcriptional complex. Pc3 and WDR-18 are in the GCN5 complex, thei directly interact with GCN5, and at least for WDR-18, it binds to the catalytic domain. These studies have provided a molecular understanding of how those two proteins migh impact hepatic glucose output. Moreover, we have identified new acetylated lysines on critical domains of PGC-1a (LxxLL. hormone nuclear receptor binding boxes). We will specifically test the importance of this new site on gluconeogenic gene expression and glucose production. Our final goal is to determine the effects of these two proteins in blood glucose levels in several animal models as described in the application. Understanding the mechanism of action of this transcriptional regulatory complex that involves a chemical modification on PGC-1α will allow us to design specific drugs that can target the activities of these proteins and efficiently control blood glucose levels that are altered in metabolic diseases and situations of high stress.

References

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Appendices

None.